Yuji MATSUZAWA, et al.
ADIPONECTIN PROMOTER AND USE...
June 27, 2005
Mark Boland 202-293-7060
Q88792
I of 9

10/540864

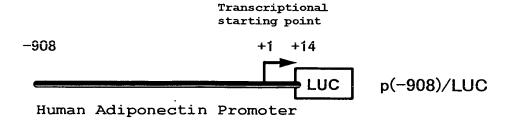
Figure 1

-908 CTTCTAGGCCAGAGCTGGGTTCCACAAGAGACAGAATAGG	-868
CATATATATGCTTAAGGAACTGGAAAAACAGGCTCTCTCT	-818
CACACACACACATACCAAGGTAGCTGTCAAAATGTTATCCGAAATTTT	-768
GGAACCAAAAAATCTTGAAAGATGGTATTCCAATATCACATTTTATGTAA	-718
GTTTTCTATTATATTAGATTCAAATTACGATTCGAGGCCACAAGCTTTAA	-668
GAATTCAGGGCCTTTTTAACTTGCCAAGCCCCACACCACTCCAGGAACTT	-618
CCCCACACCCCAGTTCTCAGAATTCATGTGCAAGGTCTTTCCTAAATCCA	-568
GGGTCCAGGTCAGAGAGTGGAGGATGTGCTCTATTTCTTACCTGATTGCA	-518
GACCCCTCTGACAGTGCTCCCTTCTGAAGCACTCACTGTCTGAACGTACA	-468
CAGTCTCAGACTTAATCATGCACAGTGAGCAAGACTGTGGTGTGATAATT	-418
GGCGTCCCTGACTTATTAGGGCAAATCTATGGGAGGGGGAGACCTCCTGG	-368
ACCACTGAGCAATTAATTCATTTACATTAGGAAGTTTCTCCGTCAGATGC	-318
AGGAAAAAATCTTGTTTTCCTGCTGTGGTTT <u>TGACTTTTGCCCC</u> ATCTT	-268
-285 -273 PPRE CTGTTGCTGTTGTAGGAGGCAAAATAAGGG <mark>TCAAGGCCT</mark> GGAAACACAAG -237 -229	-218
LRH-RE TGCTTTGACTGAAGCTCCACTTGGCTTCCGAAGCCCAAGCTGGGTTGTAC	-168
CAGGTTCCCTAGGGTGCAGGCTGTGGGCAACTGCCAGGGACATGTGCCTG	-118
CCCACCGGCCTCTGGCCCTCACTGAGTTGGCCAATGGGAAATGACAATTG	-68
TGAGGTGGGGACTGCCTGCCCCGTGAGTACCAGGCTGTTGAGGCTGGGC	-18
CATCTCCTCCTCACTTC / CATTCTGACTGCAG	+14

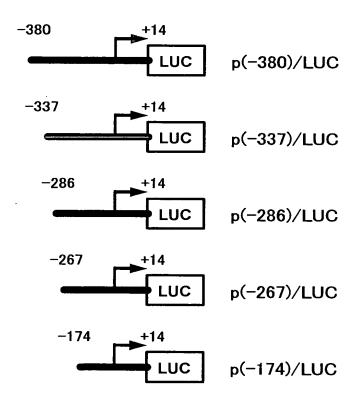
Yuji MATSUZAWA, et al.
ADIPONECTIN PROMOTER AND USE...
June 27, 2005
Mark Boland 202-293-7060
Q88792
2 of 9

10/540864

Figure 2
Structure of Human Adiponectin Promoter/Reporter Plasmid



Deletion Constructs of Human Adiponectin Promoter



Yuji MATSUZAWA, et al. ADIPONECTIN PROMOTER AND USE... June 27, 2005 Mark Boland 202-293-7060 Q88792 3 of 9

10/540864

Figure 3

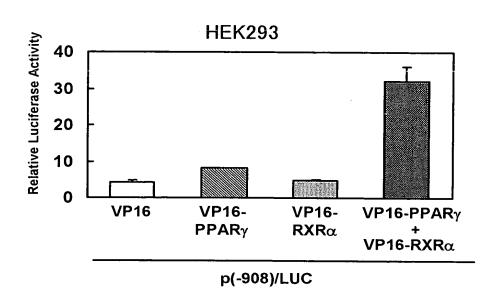


Figure 4 **HEK293** pGL3 □ VP16 ■ VP16-PPARγ + VP16-RXRα p(-908)/LUC p(-380)/LUC p(-337)/LUC p(-286)/LUC p(-267)/LUC p(-174)/LUC 5 0 10 15 20

Relative Luciferase Activity

10/540864

Yuji MATSUZAWA, et al. ADIPONECTIN PROMOTER AND USE... June 27, 2005 Mark Boland 202-293-7060 Q88792

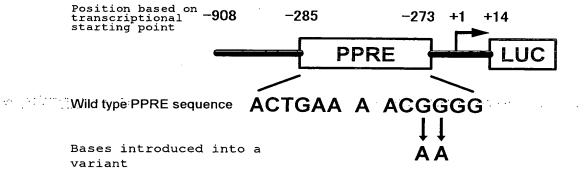
4 of 9

Figure 5

Gene	PPRE sequence
Human adiponectin	5'- GGGGCA A AAGTCA -3'
Mouse aP2	5'- GGGTGA A ATGTGC -3' 5'- GGATCA G AGTTCA -3'
Mouse c-Cbl binding protein	5'- AGGCTA A AGGTCA -3'
Mouse LXRα	5'- GGGGCA A AGTTCA -3'
Mouse aquaporin adipose	5'- AGGGGA G AGGTCA -3'

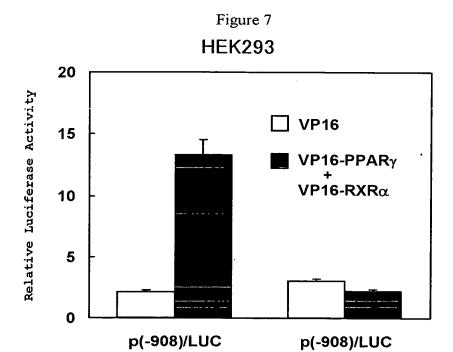
Figure 6

Human Adiponectin Promoter/Reporter



Yuji MATSUZAWA, et al.
ADIPONECTIN PROMOTER AND USE...
June 27, 2005
Mark Boland 202-293-7060
Q88792
5 of 9

10/540864



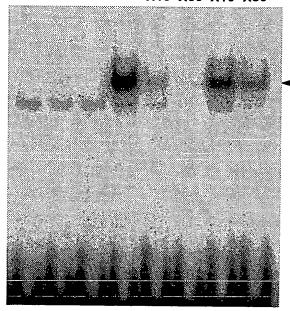
PPRE mut

Wt

Yuji MATSUZAWA, et al.
ADIPONECTIN PROMOTER AND USE...
June 27, 2005
Mark Boland 1, 202-293-7060
Q88792
6 of 9

10/540864

Figure 8



lane 1 2 3 4 5 6 7 8

Figure 9

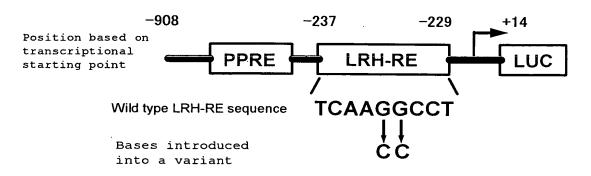
Gene	LRH-RE sequence
Human adiponectin	5'- TCAAGGCCT -3'
Rat CYP7A1	5'- TCAAGGCCG -3'
Human CYP7A1	5'- TCAAGGCCA -3'
Human CETP	5'- GCAAGGTCC -3'
Rat CYP8B1	5'- GCAAGGTCC -3' 5'- CCAAGGGCA -3'

Yuji MATSUZAWA, et al.
ADIPONECTIN PROMOTER AND USE...
June 27, 2005
Mark Boland 202-293-7060
Q88792
7 of 9

10/540864

Figure 10

Human Adiponectin Promoter/Reporter



Yuji MATSUZAWA, et al. ADIPONECTIN PROMOTER AND USE... June 27, 2005 Mark Boland 202-293-7060 Q88792 8 of 9

10/540864

Figure 12

Nuclear receptors synthesized

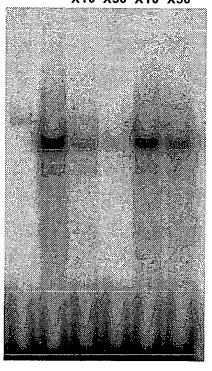
in vitro

LRH-1

LRH-RE LRH-RE

Competitive oligoDNA

wt mut X10 X50 X10 X50



lane

Yuji MATSUZAWA, et al. ADIPONECTIN PROMOTER AND USE... June 27, 2005 Mark Boland Q88792 9 of 9

202-293-7060

101540864

Figure 13

